

EFFECTS OF PROCESSING METHOD ON THE NUTRITIVE QUALITY OF GUANACASTE TREE (*Enterolobium cyclocarpum*) SEEDS

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ABSTRACT

Plants remain a significant source of food in human and animal nutrition. However, extensive inclusion of these is limited by various factors which include anti-nutrient and fibre contents which might impair their nutritional values and restrict their bio-availability. Seeds of *Enterolobium cyclocarpum* were subjected to three processing methods: autoclaving, sprouting and fermentation to evaluate the effects of these on the proximate composition and anti-nutritional property. The crude protein of the kernel meal was increased when autoclaved, sprouted and fermented relative to the raw form. There was a decrease in crude fat of the sprouted and fermented *E. cyclocarpum* kernel meal relative to the raw. The levels of the anti-nutritional factors were affected by the processing methods. There was a reduction in the phytic acid level from 100% in the raw to 16.23% (autoclaved), 10.50% (sprouted) and 10.50% (fermented). Tannins were also reduced from 100% in the raw form to 4.59% (autoclaved), 7.64% (sprouted) and 0.22% (fermentation) while saponin increased from 11.46 mg/100g in the raw form to 403.91mg/100g (autoclaved), 648.10mg/100g (sprouted) and 40.50mg/100g (fermentation). Essential amino acid profiles of the processed kernel meals were improved relative to the raw except for tryptophan which was slightly reduced by the fermentation while the processing methods increased the non-essential amino acids except for glycine and cystine which were slightly reduced by autoclaving method of processing. Mineral profiles of the test ingredient were affected by the three processing methods. All the processing methods increased Sodium, Potassium, Magnesium, Iron, Zinc and Phosphorus contents. Autoclaving improved Omega 3 and mono-saturated fatty acid while sprouting and fermentation reduced the Omega 3 and mono-saturated fatty acid value. The different processing methods used in the study were considered positive as they were effective in increasing the crude protein content of the kernel meal and reducing some of the anti-nutrients, thereby improving the nutritional value of *E. cyclocarpum* kernel meal. The study recommended that these processing methods could be of great advantage in processing *E. cyclocarpum*.

Keywords: *Enterolobium cyclocarpum*; autoclaving; sprouting; fermentation; proximate composition; anti-nutrient property ;feed ingredients

INTRODUCTION

Plant seeds are a significant source of nutrition and are frequently consumed by hu-

mans and animals. *Enterolobium cyclocarpum*, commonly known as the Guanacaste tree or the Elephant ear tree, is a large and fast-

growing tree species found in tropical regions of the Americas, including Mexico, Central America, and Northern South America. However, it is naturalized extensively in Nigeria where it exists as a fodder in experimental plots. *E. cyclocarpum* is a valuable tree species for reforestation and agroforestry due to its ability to fix nitrogen and improve soil fertility, as well as its use in the production of high-quality wood (Guzman-Mendoza *et al.*, 2019).

E. cyclocarpum has potential as a source of food and medicine in addition to its significance for the environment and economy. The seeds that are sometimes referred to as "cuajilote," have a long history of use in Mexico for a variety of purposes, including food and medicine (Garca-Guerrero *et al.*, 2018). The plant has nutritional qualities comparable to beans and other leguminous plants (Hernandez *et al.* 2014). The seeds must be treated before consumption because they contain tannins, saponins and phytic acid, as anti-nutritional substances. There haven't been many investigations into how various processing methods affect the nutritional value of the meal made from *E. cyclocarpum* kernels. However, the presence of anti-nutritional components in seed meals might impair their nutritional value and restrict their bioavailability. By lowering the amounts of anti-nutritional components and increasing the nutritional content of seed meals, processing techniques such as soaking, fermentation, hydrothermal and roasting have been employed to increase their nutritive quality (Egounlety and Awonorin, 2003; Oyeyinka *et al.*, 2019). Processing methods such as roasting, hydrothermal and soaking of plant seeds have been proven to result in higher nutritional contents and reduced levels of anti-nutritional components (Alozie *et al.*, 2016; Ayo

et al., 2020). However, the impact of different processing methods on the nutritional contents of *Enterolobium cyclocarpum* kernel meal is not yet known.

Studies have revealed that the nutritional quality of plant seed meals can be affected differently by various processing techniques. According to several studies (Akanke *et al.*, 2012; Sowmya and Jeyakumar, 2013), roasting has been shown to raise the amounts of some amino acids, minimize anti-nutritional factors and improve protein digestibility in some seed meals. On the other hand, it has been demonstrated that boiling lowers the content of several vitamins and minerals in seed meals (Fasoyiro and Fagbemi, 2007). Therefore, it is crucial to assess how different processing techniques affect the nutritional value of *E. cyclocarpum* to have a better understanding of the plant's potential as food or feed source and reveal the best processing technique for enhancing its nutritional value.

The purpose of this study was to determine the effects of different processing techniques, such as autoclaving, fermentation and sprouting on the nutritional composition of *E. cyclocarpum* kernel.

MATERIALS AND METHODS

COLLECTION OF SEEDS

Matured seeds of *E. cyclocarpum* (Plate 1) were collected during the fruiting season on the premises of the Federal University of Abeokuta, Ogun State. The seeds were collected randomly as ripe fallen fruits (pods). The fresh pods were collected in sacks and kept in the farm store at a temperature range of 26-29°C. The pods were sun-dried for five days, and dehulled manually to free the seeds. The seeds were weighed and divided into equal batches. The kernels were extract-

ed by cracking the seeds open. The batch used for germination/sprouting was tested for dormancy and viability via the Rolled Paper Towel Test (Esonu *et al.* 1998). Ten seeds of *E. cyclocarpum* were rolled up in a moistened paper towel and kept at room temperature for ten days to check for viability. On the tenth day, it was discovered that 90% of the seeds were viable. Other processing methods used included autoclaving and fermentation (solid-state fermentation).

AUTOCLAVING

2500 g of the seed kernel was cleaned off the debris and weighed out on an electronic digital weighing balance (GOLDEN-METTLER U.S.A, Model: HC-D). The kernel was wrapped in an aluminum foil and was autoclaved at 120°C for 15 minutes at 15 PSI (pound per square inch) in an 18L Laboratory Autoclave (Gallenkomp Autoclave, Model: YX-280A). This was oven-dried at 60°C for 6 hours, milled into powdery form, and stored at 20°C in a refrigerator.

FERMENTATION

The solid-state fermentation procedure described by Ruskin and Djen (1974) was employed. A total of 2.5 kg of the *Enterolobium cyclocarpum* kernel was milled into smaller particulate sizes. The milled sample was autoclaved in an 18 L laboratory autoclave at 120°C for 15 minutes at 15 PSI (pounds per square inch) to disinfect and eliminate microbial contaminants. After cooling, the material was packed into thick cellophane bags. Then, 2.0 kg of the autoclaved kernel

meal was measured and thoroughly mixed with 2 litres of sterile distilled water containing 20 mL of broth culture with a concentration of 3.0×10^8 CFU/mL of *Lactobacillus plantarum*. This was placed in Seradon Incubator, Model DHP-9052 for fermentation at 36°C for 72 hours; the product of fermentation was oven dried at a temperature of 60°C for 24 hours and milled into powdery form and stored at 20°C in a refrigerator.

GERMINATION

Germination was initiated by soaking 7 kg of the seed with water at room temperature for 24 hours. A black plastic 35-litre basin was filled with 20 kg of soil which was moistened with 5 liters of water. Afterwards, a sack was spread on the moistened soil in the basin and the soaked seeds were spread on the sack in the basin, and covered with a sack and another 35-litre plastic basin to exclude light, kept at room temperature (25°C) and allowed to germinate for five days. The essence of moistening the soil was to provide moisture during the sprouting period. Germination in this study was achieved by the emergence of the plumule and radicle (Figure 2); germination was terminated by dipping the sprouted seeds in boiling (100°C) water for 1 minute.

Thereafter, the seed coats of the germinated seeds were removed manually; then oven-dried for 24 hours at a temperature of 60°C, and milled into powdery and homogenous particulate size using a locally-fabricated grinding machine.



Plate 1: *Enterolobium cyclocarpum* fruit/pod, seed and kernel



Plate 2: Germinated seeds and kernels of *Enterolobium cyclocarpum*

PROXIMATE ANALYSES

The Proximate compositions of the processed (Raw, Autoclaved, Fermented and sprouted) *E. cyclocarpum* kernel meals were analysed according to AOAC (2005) methods. Analyses of anti-nutritional factors (Tannin, Saponins and Phytic acid) were determined according to the methods described by Ejikeme *et al.* (2014) on the raw, autoclaved, fermented and sprouted *E. cyclocarpum* kernel meals to assess the effectiveness of the processing methods in reducing the anti-nutritional contents of the kernel meals.

The amino acid profile, fatty acid profile and mineral profile of the processed *E. cyclocarpum* kernel meals were analyzed using Near Infra-Red Spectroscopy (NIRS), an instrument of FOSS analyser DS 2500 installed with software package WinISI II.

Data Collection, Analysis, and Mean Separation

Data collected from the laboratory analyses included proximate composition (moisture, crude protein, crude fat, ash, crude fibre, and nitrogen-free extract), anti-nutritional factors (phytic acid, tannins, and saponins), amino

acid profile (essential and non-essential), fatty acid profile, and mineral content (e.g., sodium, potassium, magnesium, iron, zinc, phosphorus, etc.) of the differently processed *Enterolobium cyclocarpum* kernel meals. All quantitative data were subjected to statistical analysis using Analysis of Variance (ANOVA) to determine the effects of the various processing methods (autoclaving, sprouting, and fermentation) on the nutritional and anti-nutritional parameters of the kernel meals. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0.

Where significant differences were detected ($p < 0.05$), means were separated using Duncan's Multiple Range Test (DMRT). The results were presented as mean \pm standard deviation (SD), and differences among treatments were denoted by different superscript letters in the same row across treatment groups.

RESULTS

Proximate and Antinutrients components of differently processed *E. cyclocarpum* kernel meals

Autoclaving, sprouting and fermentation increased the crude protein content of the raw kernel meal from $33.24 \pm 5.20\%$ (raw kernel) to 36.51 ± 4.30 , 37.69 ± 2.80 and $36.83 \pm 3.05\%$, respectively (Table 1). The crude fat of the raw kernel ($2.87 \pm 0.05\%$) was reduced to $1.82 \pm 0.30\%$ and $2.20 \pm 0.02\%$ by sprouting and fermentation, respectively while autoclaving increased the crude fat to $3.01 \pm 0.50\%$. Moisture content ($10.60 \pm 1.05\%$) of the raw kernel meal was reduced by autoclaving, sprouting and fermentation to 10.30 ± 0.89 , 9.80 ± 0.90 and $9.32 \pm 0.60\%$, respectively. Ash content was slightly increased from $2.68 \pm 0.50\%$ in raw kernel meal by auto-

claving, sprouting and fermentation to 2.96 ± 0.10 , 3.21 ± 0.60 and $3.97 \pm 0.85\%$, respectively. Crude fibre increased with the three processing methods from 2.22% in the raw kernel meal to 2.33 ± 0.00 , 2.86 ± 0.80 and $2.68 \pm 0.01\%$ by autoclaving, sprouting and fermentation, respectively. Processing methods (autoclaving, sprouting and fermentation) reduced the NFE of the kernel meal from 48.39 ± 3.00 in raw to 44.89 ± 2.50 and $45.00 \pm 1.10\%$, respectively. Phytic acid was reduced from 4.19 ± 0.30 mg/100 g in the raw kernel meal to 0.68 ± 0.02 mg/100g in autoclaving, 0.44 ± 0.00 mg/100 g in sprouting and 0.44 ± 0.10 mg/100 g in fermentation. Tannins reduced from 11050.43 ± 25.20 mg/100 g in the raw kernel meal to 507.00 ± 15.80 mg/100 g, 844.50 ± 23.05 mg/100 g and 24.37 ± 4.30 mg/100 g, while saponins increased from 11.46 ± 0.05 mg/100 g in the raw kernel meal to 403.91 ± 8.45 mg/100 g, 648.10 ± 10.20 mg/100 g and 40.50 ± 2.3 mg/100 g in autoclaving, sprouting and fermentation, respectively (Table 1).

There was significant difference among the means of crude protein, crude fat NFE, phytic acid, tannins and saponins.

Amino acid profiles of differently processed *Enterolobium cyclocarpum* kernel meal

Essential amino acid profiles of the differently processed *E. cyclocarpum* kernel meal were greatly increased by the three processing methods (Table 2). Histidine was increased by autoclaving, sprouting, and fermentation from 0.86 ± 0.01 g/100 g in the raw kernel meal to 0.88 ± 0.05 g/100g, 0.90 ± 0.00 g/100 g and 0.88 ± 0.03 g/100 g, respectively. Arginine increased from 2.73 ± 0.03 g/100 in raw kernel meal to 2.80 ± 0.01 g/100g, 2.86 ± 0.03 g/100g and

Table 1: Proximate composition and anti-nutritional factors of differently processed *Enterolobium cyclocarpum* kernel meals

Parameter	Raw	Autoclaved	Sprouted	Fermented
Moisture (%)	10.60 ± 1.05	10.30 ± 0.89	9.80 ± 0.90	9.32 ± 0.60
Protein (%)	33.24 ± 5.20 ^c	36.51 ± 4.30 ^b	37.69 ± 2.80 ^a	36.83 ± 3.05 ^b
Crude fat (%)	2.87 ± 0.05 ^b	3.01 ± 0.50 ^a	1.82 ± 0.30 ^d	2.20 ± 0.02 ^c
Crude fibre (%)	2.22 ± 0.02	2.33 ± 0.00	2.86 ± 0.80	2.68 ± 0.01
Ash (%)	2.68 ± 0.50	2.96 ± 0.10	3.21 ± 0.60	3.97 ± 0.85
NFE (%)	48.39 ± 3.00 ^a	44.89 ± 2.50 ^b	44.62 ± 1.60 ^b	45.00 ± 1.10 ^b
Phytic acid (mg/100g)	11,050.43 ± 25.20 ^a	507.00 ± 15.80 ^c	844.50 ± 23.05 ^b	24.37 ± 4.30 ^d
Tannins (mg/100g)	11.46 ± 0.85 ^d	403.91 ± 8.45 ^b	648.10 ± 10.20 ^a	40.50 ± 2.34 ^c
Saponins (mg/100g)	4.19 ± 0.30 ^a	0.68 ± 0.02 ^{bc}	0.44 ± 0.00 ^c	0.44 ± 0.10 ^c

Mean values with different superscripts along the same row are significantly different (p<0.05). NFE=Nitrogen Free Extract

2.81±0.03 g/100 in autoclaving, sprouting, and fermentation, respectively. Autoclaving, sprouting, and fermentation increased the threonine value from 1.13±0.05g/100g in raw kernel to 1.14±0.02 g/100g, 1.17±0.10 g/100g and 1.15±0.01 g/100 g, respectively. Valine was increased by autoclaving, sprouting, and fermentation from 1.42±0.01 g/100g in the raw kernel meal to 1.47±0.00 g/100g, 1.47±0.03 g/100g and 1.48±0.02 g/100g, respectively. Methionine value was not influenced by fermentation but increased 0.34±0.01 g/100g in the raw kernel to (0.35±0.05 and 0.37±0.08) g/100g in autoclaving and sprouting, respectively. Autoclaving increased the lysine value from 1.48±0.02 g/100g in raw kernel meal to 1.53±0.02 g/100g and was increased in the same manner to (1.54±0.01 and 1.50±0.03) g/100g by sprouting and fermentation. Isoleucine and leucine values were increased. Autoclaving, sprouting, and fermentation increased the isoleucine value from 1.14±0.01g/100 g in the raw kernel meal to (1.19±0.50, 1.15±0.04 and 1.16±0.05) g/100g respectively. Leucine increased from

1.49±0.20 g/100 g in raw kernel meal to (1.55±0.05, 1.51±0.03 and 1.67±0.03) g/100 g in autoclaving, sprouting and fermentation, respectively. Autoclaving, sprouting and fermentation increased the phenylalanine value from 1.57±0.30 g/100g in the raw kernel meal to (1.58±0.05, 1.59±0.05 and 1.59±0.01) g/100 g, respectively. Tryptophan value was slightly reduced by fermentation from 0.35±0.01 g/100g in the raw kernel meal to 0.33±0.01 g/100g and increased to 0.36±0.00 g/100g by autoclaving and sprouting (Table 2).

Autoclaving, sprouting and fermentation increased the aspartic acid from 3.65±0.50 g/100g (in the raw kernel meal) to 3.70±0.40 g/100g, 3.74±0.40 g/100g and 3.66±0.20 g/100g, respectively (Table 2). Serine was increased from 1.80±0.10 g/100g in the raw kernel meal to 1.82±0.09 g/100, 1.86±0.03 g/100g and 1.85±0.20 g/100g by autoclaving, sprouting and fermentation, respectively. Glutamic acid increased from 3.14±0.05 g/100g in raw kernel meal to 3.15±0.04 g/100g, 3.60±0.04 g/100g and

3.58±0.05 g/100g in autoclaving, sprouting and fermentation, respectively. Glycine was reduced by autoclaving from 1.25±0.00 g/100g in raw kernel to 1.22±0.10 g/100g and increased to 1.35±0.02 g/100g and 1.42±0.10 g/100g in sprouting and fermentation. The processing methods improved alanine value from 1.03±0.03 g/100g in raw kernel meal to (1.04±0.00, 1.08±0.02 and 1.16±0.01) g/100g in autoclaving, sprouting and fermentation. Proline value also was increased from 1.18±0.02 g/100g in the raw kernel meal to (1.27±0.05, 1.29±0.03 and 1.30±0.01) g/100g in autoclaving, sprouting and fermentation respectively. Cystine value was decreased by autoclaving from 0.58±0.00 g/100g in raw kernel meal to 0.49±0.00 g/100g and increased to 0.60±0.01 and 0.60±0.06 g/100g in sprouting and fermentation. Tyrosine value increased from 1.02±0.05 g/100g in raw kernel meal to (1.04±0.01, 1.09±0.01 to 1.05±0.03) g/100 g in autoclaving, sprouting and fermentation respectively. There was significant difference among the means of leucine (Table 2)

Table 2: Amino acid profiles of differently processed *Enterolobium cyclocarpum* kernel meals

Parameters (g/100 g)	Raw	Autoclaved	Sprouted	Fermented
Essential				
Histidine	0.86±0.01	0.88±0.05	0.90±0.00	0.88±0.03
Arginine	2.73±0.03	2.80±0.01	2.86±0.03	2.81±0.03
Threonine	1.13±0.05	1.14±0.02	1.17±0.10	1.15±0.01
Valine	1.42±0.01	1.47±0.00	1.47±0.03	1.48±0.02
Methionine	0.34±0.01	0.35±0.05	0.37±0.05	0.34±0.00
Lysine	1.48±0.02	1.53±0.02	1.54±0.01	1.50±0.03
Isoleucine	1.14±0.01	1.19±0.50	1.15±0.04	1.16±0.05
Leucine	1.49±0.20 ^b	1.55±0.05 ^{ab}	1.51±0.03 ^{ab}	1.67±0.03 ^a
Phenyl alanine	1.57±0.30	1.58±0.05	1.59±0.05	1.59±0.01
Tryptophan	0.35±0.01	0.36±0.00	0.36±0.01	0.33±0.01
Non-essential				
Aspartic acid	3.65±0.50	3.70±0.40	3.74±0.40	3.66±0.20
Serine	1.80±0.10	1.82±0.09	1.86±0.03	1.85 ±0.20
Glutamic acid	3.14±0.05	3.15±0.04	3.60±0.04	3.58±0.05
Glycine	1.25±0.00	1.22±0.10	1.35±0.02	1.42±0.10
Alanine	1.03±0.03	1.04±0.00	1.08±0.02	1.16±0.01
Proline	1.18 ±0.02	1.27±0.05	1.29±0.30	1.30±0.01
Cystine	0.58±0.00	0.49±0.00	0.60±0.01	0.60±0.06
Tyrosine	1.02±0.05	1.04±0.01	1.09±0.01	1.05±0.03

Mean values with different superscripts along the same row are significantly different (p<0.05).

Mineral profile of the differently processed *Enterolobium cyclocarpum* kernel

Sodium (Na) in the raw kernel increased from 1239.86±80.50 ppm to 1806.87±50.20 ppm, 1443.27±80.60 and 1248.97±60.50 ppm by autoclaving, sprouting and fermentation, respectively. The three processing methods increased the potassium (K) value from 8130.64±50.00 ppm in the raw kernel meal to 10,946.28±100.50 ppm, 9131.88±90.50 ppm and 8680.09±85.60 ppm in autoclaving, sprouting and fermentation, respectively. Magnesium (Mg) was increased from 2227.44±50.00 ppm in the raw kernel meal to 2425.67±80.50 ppm, 2552.60±90.50 ppm and 2661.08±85.60 ppm in autoclaving, sprouting and fermentation, respectively. Fermentation reduced the manganese (Mn) level in the raw kernel meal from 38.88±2.50 ppm to 24.33±0.50 ppm and was increased to 64.00±5.30 ppm and 41.75±6.10 ppm by autoclaving and sprouting. Iron (Fe) was increased by the processing methods. Fe increased from

830.38±20.25 ppm in the raw kernel meal to 840.51±20.20 ppm in fermentation, 1267.29±40.20 ppm in sprouting and 1429.83±80.50 ppm in autoclaving. Cu value was decreased from 14.23±0.50 ppm in the raw kernel by autoclaving (10.92±1.00 ppm) and sprouting (12.09±1.50 ppm) and was increased by fermentation (14.77±1.90 ppm). Zinc (Zn) was increased by the different processing methods. The value increased from 32.98±4.55 ppm in the raw kernel meal to (37.49±4.20, 46.54±4.30 and 44.96±5.20) ppm in autoclaving, sprouting and fermentation, respectively. The different processing methods increased the phosphorus (P) value from 2764.02±100.50 ppm in the raw kernel meal to 3,848.69±180.00 ppm, 4,162.46±150.20 ppm 4260.81±190.80 ppm in autoclaving, sprouting and fermentation, respectively.

There was significant difference among the means of the mineral profile of the differently processed *E. cyclocarpum* kernel meal.

Table 3: Mineral profile of differently processed *Enterolobium cyclocarpum* kernel meal (ppm)

Parameters (ppm)	Raw	Autoclaved	Sprouted	Fermented
Sodium	1,239.86 ± 80.50 ^c	1,806.87 ± 50.20 ^a	1,443.27 ± 80.60 ^b	1,248.97 ± 60.50 ^{bc}
Potassium	8,130.64 ± 90.50 ^d	10,946.28 ± 100.50 ^a	9,131.88 ± 90.50 ^b	8,680.09 ± 85.60 ^c
Magnesium	2,227.44 ± 50.00 ^d	2,425.67 ± 80.50 ^c	2,552.60 ± 90.50 ^b	2,661.08 ± 85.60 ^a
Manganese	38.88 ± 2.50 ^c	64.00 ± 5.30 ^a	41.75 ± 6.10 ^b	24.33 ± 0.50 ^d
Iron	830.38 ± 20.25 ^c	1,429.83 ± 80.50 ^a	1,267.29 ± 40.20 ^b	840.51 ± 20.20 ^c
Copper	14.23 ± 0.50 ^a	10.92 ± 1.00 ^c	12.09 ± 1.50 ^b	14.77 ± 1.90 ^a
Zinc	32.98 ± 4.55 ^c	37.49 ± 4.20 ^b	46.54 ± 4.30 ^a	44.96 ± 5.20 ^a
Phosphorus	2,764.02 ± 100.50 ^c	3,848.69 ± 180.00 ^b	4,162.46 ± 150.20 ^a	4,260.81 ± 190.80 ^a

Mean values with different superscripts along the same row are significantly different (p<0.05).

Fatty acid profile of differently processed *Enterolobium cyclocarpum* kernel meal

Omega 3 fatty acid in the raw kernel meal reduced from 59.88 ± 5.05 mg/100 g to 50.07 ± 2.50 mg/100 g and 56.66 ± 4.86 mg/100 g in sprouting and fermentation, respectively and increased to 60.29 ± 4.30 mg/100 g in autoclaving. Sprouting and fermentation reduced the trans-fatty acid from 13.52 ± 2.3 mg/100 g in raw kernel meal to 12.62 ± 3.50 mg/100 g and 13.44 ± 2.30 mg/100 g, respectively and increased to 14.84 ± 2.80 mg/100 g by autoclaving. Processing decreased the saturated

fatty acid from 4345.04 ± 70.50 mg/100 g in the raw kernel meal to 4031.81 ± 80.30 mg/100 g in sprouting and 4336.84 ± 68.50 mg/100 g in fermentation and increased to 4674.38 ± 20.50 mg/100 g in autoclaving. Unsaturated fatty acid was decreased from 1420.56 ± 50.20 mg/100 g in raw kernel meal to $(1166.62 \pm 70.50$ and $1175.18 \pm 80.50)$ mg/100 g in Sprouting and fermentation, respectively and increased to 1755.69 ± 59.30 mg/100 g by autoclaving.

There was a significant difference among the means of the fatty acid profile of the differently processed *E. cyclocarpum* kernel meal.

Table 4: Fatty acid profile of differently processed *Enterolobium cyclocarpum* kernel meal

Parameter (mg/100 g)	Raw	Autoclaved	Sprouted	Fermented
Omega 3 fatty acid	59.88 ± 5.05^a	60.29 ± 4.30^a	50.07 ± 2.50^c	56.66 ± 4.86^b
Trans fatty acid	13.52 ± 2.3^b	14.84 ± 2.80^a	12.62 ± 3.50^c	13.44 ± 2.30^{bc}
Saturated fatty acid	$4,345.04 \pm 70.50^b$	$4,674.38 \pm 20.50^a$	$4,031.81 \pm 80.30^c$	$4,336.84 \pm 68.50^b$
Unsaturated fatty acid	$1,420.56 \pm 50.20^b$	$1,755.69 \pm 59.30^a$	$1,166.62 \pm 70.50^c$	$1,175.18 \pm 80.50^c$

Mean values with different superscripts along the same row are significantly different ($p < 0.05$).

DISCUSSION

The three processing methods: autoclaving, sprouting and fermentation improved the nutritional value of the *Enterolobium cyclocarpum* kernel meals and reduced some of the anti-nutritional factors specifically, tannin and phytic acid. The crude protein content of the *Enterolobium cyclocarpum* kernel meal increased significantly following autoclaving, sprouting, and fermentation. This enhancement can be attributed to processing-induced reductions in anti-nutritional factors, particularly phytate, which may otherwise inhibit protein availability. The increase in crude protein content

observed in this study aligns with earlier findings on other leguminous seeds. For instance, Adegunwa *et al.* (2012) reported similar protein increases in Bennisseed (*Sesamum indicum*) following thermal processing, while Kaushik *et al.* (2010) and Kayemba and Van Rensburg (2013) noted protein improvements in germinated soybeans. Although the crude protein content recorded here was slightly lower than values reported for some commonly used legumes such as soybean (40–45%) and comparable to mung bean (23–33%) as reported by Mubarak (2005), it demonstrates that *E. cyclocarpum* can be a valuable protein source when appropriately pro-

cessed. This was in line with the observation of Adegunwa *et al.*, (2012) who noted that thermal processing results in significant reductions in the content of anti-nutritional factors (oxalate and phytate) of Bennisseed. On the other hand, the increase in protein content and decrease in crude fat of the sprouted *E. cyclocarpum* kernel meal could be attributed to the utilization of carbohydrates and fat as energy sources for developing sprouts. This finding is consistent with previous reports. Mwikya *et al.* (2000) observed a gradual decrease in starch content in finger millet during the first 36 hours of germination, while Mubarak (2005) similarly reported a reduction in starch content in mung bean seeds after three days of germination. In contrast, Kaushik *et al.* (2010) found that germination led to an increase in crude protein content in soybeans; a trend further supported by Kayemba and Van Rensburg (2013), who reported a progressive increase in crude protein as the duration of soybean germination increased. These observations suggest that germination not only reduces carbohydrate reserves but also enhances protein concentration, likely due to metabolic reorganization and the synthesis of enzymes and structural proteins during early seed development. Kajila *et al.*, 2017 suggested that germination as a catabolic process entails an increase in the activities of lipolytic enzymes and lipases that convert the fats and oil, into fatty acid, glycerol and carbohydrates to glucose respectively. This process might lead to the breaking down of the seed carbohydrate into simple fats which the embryo uses as its source of growth during sprouting.

The decrease in crude fat and increase in the percentage of crude protein of fermented *E. cyclocarpum* kernel meal could be due to microbes using lipids as an energy source

and other microbial activities during fermentation. One of such activities (according to Steinkraus, 1995; Wikipedia, 2016) is the biological enrichment of food substrate with protein, essential amino acids and vitamins. Mukherjee *et al.*, (2016), observed that enhanced crude protein content could be due to the increase in the microbial biomass i.e. biomass of the fermenting organism.

The increase in crude fibre of the fermented *E. cyclocarpum* in this study was in line with the studies of Torres *et al.*, (2006) who reported that fermentation of *Mucana sloanei*, *Detarium microcarpum* and pigeon pea also revealed an increase in protein and crude fibre contents and contrary to the findings of Martins-Carbeja *et al.*, (2009) who reported that fermentation decreased the soluble dietary fibre and cellulose content of beans.

The levels of the anti-nutritional factors that were drastically affected in this study could be attributed to the various processing methods. According to the results, sprouting and fermentation showed greater effectiveness in reducing phytic acid content compared to autoclaving. This indicates that all three processing methods contributed positively to improving the nutritional quality of *E. cyclocarpum* seeds. These findings are in line with those of Rehman and Shah (2005), Shimelis and Rakshit (2007), Vadivel *et al.* (2008), Lou *et al.* (2009), and Doss *et al.* (2011), who also reported that processing techniques such as sprouting, fermentation, and thermal treatment significantly reduced anti-nutritional factors like phytic acid and tannins in various legume seeds, thereby enhancing their nutritional quality and mineral bioavailability. The germination and fermentation processes that reduced the anti-nutritional content (phytic acid and tannin) in this study corroborate the reports of Ibra-

him *et al.* (2005), Idris *et al.* (2005), and Hussain *et al.* (2011), who observed that such physiological and processing methods significantly decrease anti-nutritional factors in legume seeds, thereby enhancing their nutritional quality and mineral bioavailability.

However, these processing methods were not potent in reducing the saponin content of the kernel meals of *E. cyclocarpum*. They were elevated when compared to the value in the raw form. Anderson and Wolf (1995) stated that saponins could be degraded by acid and alkaline hydrolysis while Gestetne *et al.* (1968) reiterated that saponins could as well be degraded by bacterial glucosidases.

The different processing methods improved the essential amino acid profiles of the kernel meal, except for tryptophan, which was slightly reduced by fermentation. The processing methods also increased the non-essential amino acid content, except for glycine and cystine, which were slightly reduced by the autoclaving method. This increase could be due to the impact of the different processing methods on the amino acids profile of the test ingredient. Amino acids are the synthesizing units of protein and this increase in the amino acid values gave rise to the increase in the crude protein values of the autoclaved, sprouted and fermented products. Similarly, the mineral profiles of the test ingredient were affected by the three processing methods. Sodium (Na), Potassium (K), Magnesium (Mg), Iron (Fe), Zinc (Zn) and Phosphorus (P) were increased by all the processing methods. According to FAO (2014), several limiting factors, such as high fibre content, poor digestibility, and the presence of anti-nutritional factors (including lectins, trypsin inhibitors, saponins, tannins, genistein, and

protease inhibitors), as well as high phytic acid content, can bind metal ions such as magnesium, calcium, phosphorus, manganese, iron, and zinc, thereby rendering them unavailable for absorption. Nkhata, *et al.*, (2018) stated that potassium is an integral part of phytate molecules where it is covalently bonded, rendering it inaccessible by digestive enzymes. These processing methods might have broken the covalent bond binding these metal ions of the test ingredient which led to the increase in the sodium, potassium, magnesium, iron, zinc, and phosphorus contents of the processed kernel meal. This could be a result of these minerals in the raw form being locked up by the anti-nutritional factor as phytate. These processing methods might have broken the structural integrity of the phytate thereby releasing or freeing the phosphorus and other metals such as Magnesium, Sodium, Zinc, Iron, Potassium which led to a consequent increase in the minerals of the processed kernel meal. According to Gupta *et al.*, 2015, over 80% of the phosphorus in plants resides as phytic acid. The increase in the phosphorus, Na, K, Mn, and Fe content of the autoclaved and sprouted kernel meal could be attributed to the effects of the processing methods by breaking down of the structural integrity of proteinous inhibitors such as phytate as suggested by Adegunwa *et al.*, (2012). A similar increase was reported by Azeke *et al.*, (2011) for the fermentation of legumes. Therefore, these processing methods; autoclaving, germination and fermentation improved the mineral compositions of the processed *E. cyclocarpum* kernel meal for effective nutrient utilization. Phytic acid or phytate are known to form complexes with metal ions and as such inhibit their absorption and reduces their biological availability. Autoclaving improved Omega 3 fatty acid of the *E. cyclocarpum* kernel meal while sprouting

and fermentation reduced the Omega 3 fatty acid value. Similarly, autoclaving improved the mono-saturated fatty acid while sprouting and fermentation reduced the mono-saturated fatty acid. The reason for these reductions in the lipid profiles of the sprouted and fermented products could be due to the utilization of fat as the energy source for developing sprouts and microbial multiplication in fermented products.

Conclusively, the different processing methods are considered to be good as they were effective in increasing the crude protein composition of the kernel meal and reducing some of the anti-nutrients thereby improving the nutritional value of *E. cyclocarpum*. It is therefore recommended that either of these processing methods can be employed in the processing of *E. cyclocarpum* for better utilization of the ingredient.

CONCLUSION

This study demonstrated that autoclaving, sprouting, and fermentation are effective processing methods for enhancing the nutritional quality of *Enterolobium cyclocarpum* kernel meal. These methods significantly increase the crude protein content, improve the mineral composition, and reduce anti-nutritional factors such as tannins and phytic acid, thereby making the kernels more suitable for consumption. Autoclaving is particularly effective in improving the lipid profile while sprouting and fermentation offer a superior reduction of phytic acid. However, none of the methods are effective in reducing saponin content, which remain elevated.

The findings of this study underscore the potential of these processing techniques to improve the utilization of *E. cyclocarpum* as a viable nutritional resource for feed formula-

tion.

Future studies could focus on optimizing these methods further to address limitations such as the persistence of saponins, and to explore their applicability to other underutilized plant-based feed ingredients.

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